measuring both force and torque, has been developed as a versatile setup in tackling biologically relevant issues at high spatial and temporal resolution. Torque control in this optical tweezers setup relies on the manipulation and readout of the polarization state of light used to trap nano-fabricated birefringent cylinders. The flagellar motor of *Escherichia coli* is a well-know rotary motor of only about 45 nm embedded in the cellular membrane, but besides its protein content the exact functioning of this intriguing motor remains unknown. The rotary motor consists of a rotor attached to a flagellum and of stators 'pushing' the rotor around. Stators diffuse in the cytoplasmic membrane upon engaging in the motor complex. The temporal resolution of our setup allows to investigate fast stator dynamics. We are studying the response of the motor at stall torque, forward rotation and backward rotation by optically adjusting the load torque on the motor, on which we present preliminary results. Deploying our optical setup we are trying to unravel the mechanism by which this molecular motor works to propel bacteria.

Platform: Voltage-gated K Channels: Gating

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The Role of the 2nd Threonine Within the TTVGYGD Sequence of Kv-Channels in C-Type Inactivation Gating, Ion Selectivity and Permeation

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An allosteric communication between the activation gate (AG) in K-channels and their selectivity filter (SF) promotes the collapsing of the former, prompting the channel to enter the C-type inactivated state (OI). The 2nd Threonine within the TTVGYGD sequence of Kv-channels (2T) could be a key component for this allosteric coupling[1]. Here we report that an Alanine substitution of 2T prevents all human delayed rectifier Kv-channel subtypes (Kv1.5, Kv 2.1 and Kv3.1), the Shaker as well as the KcsA channels, from entering the (OI). Prolonged depolarization or pH activation causes Kv and KcsA channels respectively to enter (OI). However, the T to A substitution yielded non-inactivating channels that instead displayed a second (slow) activation process, which suggested the conversion of the (OI) into a second conductive state (O2). This hypothesis was confirmed by solving the X-ray structures of the KcsA-T75A mutant in the closed (C) and in the open states (O) ≅ 3.2 Å and 2.3 Å resolution respectively. The KcsA-T75A SF in the (C) look like an inactive conformation, with K+ ions bound at sites 1 and 3; while in the (O2) resembled a conductive one, with K+ ions bound at sites 1,2 and 3. This state dependent SF ion configuration fits the observation that upon activation the mutant channels gradually converted from a low conductive or inactive state into a high-second conductive (O2) state. The (O2) (SF) structure suggested a reduced single channel conductance when compared to WT, which agree with up to 10-fold drop in channel conductance recorded among the mutant channels. Interestingly, all Alanine mutants remained highly K+ selective.

1.Cuello, L.G., et al. Nature, 2010.

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Molecular Determinants of Slow Inactivation in Voltage-Gated Potassium Channels

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Potassium channels respond to prolonged depolarizations with structural rearrangements that result in inactivated, nonconducting channels through a process termed slow inactivation. Significant efforts to understand this process have been made using structural, theoretical and experimental approaches yet the molecular details of slow inactivation in eukaryotic voltage-gated potassium (Kv) channels remain poorly understood. Data gleaned from prokaryotic (KcsA) and eukaryotic (Kv1.2/2.1) channels have implicated two adjacent and highly conserved aromatic side chains near the selectivity filter as critical determinants for slow inactivation. In particular, the indole nitrogens of both side chains, Trp434 and Trp435 in Shaker potassium channels, have been proposed to contribute to a hydrogen bond network that modulates slow inactivation, yet the direct demonstration of this notion is missing in Kv channels. Here we incorporate unnatural derivatives of Trp to directly demonstrate that the indole nitrogen of Trp434 is a crucial component of the slow inactivation process through two complementary substitutions. By subtly increasing the acidity of the indole nitrogen through fluorination (and therefore strengthening its hydrogen bond donor ability), the rate of slow inactivation was substantially decreased (4-fold slower than WT). Conversely, the novel unnatural amino acid side chain Ind, which lacks the indole nitrogen but is otherwise isosteric to Trp, increased the rate of inactivation more than 10-fold when Ind-containing channels were co-expressed with WT channels, as Ind-containing channels alone did not produce ionic current. In contrast to Trp434, the indole nitrogen of Trp435 does not contribute to slow inactivation as even relatively nonconservative mutations to Phe or Tyr at this site do not affect slow inactivation. Taken together, these results directly demonstrate the functional importance of the H-bonding ability of Trp434 in open pore stability in Kv channels.

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Phe233 in the Voltage-Sensor is Rate Limiting for Channel Closure but not for the Opening

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During activation, the charged S4 segment in the voltage sensor domain (VSD) of voltage-gated ion channels is required to translate across a hydrophobic zone. This constitutes a thin aperture clearly separating the open/relaxed and closed/resting states. The nature of this barrier is critical for channel function, and has been the focus of much attention. Previously, we identified the single preserved residue F233 (F290 in Shaker) as a structural barrier for the gating charges, that uniquely determines intermediate state and substitutions modulate the deactivation barrier. A fundamental understanding of the S4 activation/deactivation barrier as well as kinetics is an important remaining challenge to decipher gating. Here, we study the free barrier and kinetics of the VSD through combining in-vitro and in-silico experiments. We used site-directed mutagenesis and measured the voltage-dependence in-vitro to study the effect of F290L compared to the wild-type (WT). In parallel, molecular dynamics simulations allowed us to identify residues interacting with the phenyl ring. Through in-silico mutations we show their impact on the barrier, as well as the structural and kinetics effects for the phenyl ring orientation during the first step of deactivation and the (reverse) last step of the activation. Strikingly, the channel closing transition shows a huge speedup from the F290L mutant (1ms, WT 10ms), in contrast to the opening that is completely unaffected. This indicates that F233 is only rate-limiting for the channel closure, but not opening and suggests different kinetics for the activation/deactivation barriers. Additionally the ring is clearly stabilized through vdW interactions with surrounding hydrophobic residues, and appears to always open by upward rotation both for activation/deactivation. This upward rotation suggests a model where the closing is possibly a mostly entropic process, while opening would be largely enthalpic.

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Movement of the S4-S5 Linker of KvAP during Gating

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Voltage-gated potassium channels are formed by four voltage sensor domains (VSD) and a central pore domain. The voltage sensors are activated during membrane depolarization, leading to pore opening through a process called electromechanical coupling. Functional and structural evidence suggests that the covalent link between the VSD and the pore, the α-helical S4-S5 linker, plays a crucial role in the electromechanical coupling. However, no data on the rearrangements at the cytosolic face of the channel is presently available leaving the molecular mechanism of coupling subject for speculation. In this study, we determined the position of the S4-S5 linker in the open and closed state of the bacterial KvAP channel. To this end, we utilized LRET (Lanthanide Resonance Energy Transfer) to measure the cross pore distances of various positions in the S4-S5 linker. A KvAP dimer with a single cysteine has been constructed resulting in a tetramer with two symmetric cysteines, which we label with a Tb-chelate and an organic fluorophore, respectively. The labeled protein is reconstituted in lipid vesicles. We have measured distances in both closed and open state for 8 positions along the 10 amino acid linker. Based on the results we aim to create a 3D model of the movement of the S4-S5 linker during the opening of the pore.

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Structural Insights into Calmodulation of Neuronal KCNQ Channels

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Calmodulin (CaM) is a ubiquitous intracellular calcium sensor for many potassium channels. For the voltage-gated KCNQ family of potassium channels, CaM binds to the intracellular C-terminus to mediate channel assembly,